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Detection of *Mycobacterium leprae* infection in wild nine-banded armadillos (*Dasypus novemcinctus*) using the rapid ML Flow test

Detecção de infecção pelo *Mycobacterium leprae* em tatus selvagens da espécie *Dasypus novemcinctus* utilizando o teste rápido ML Flow

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ABSTRACT

Mycobaterium leprae *infection was investigated in armadillos from the State of Espírito Santo, Brazil. The ML Flow test was performed on 37 nine-banded armadillos and positive results were found in 11 (29.7%). The ML Flow test may be used to identify possible sources of* Mycobaterium leprae *among wild armadillos.*

Key-words: *Leprosy. ML Flow test.* ***Reservoir. Armadillos.***

RESUMO

Tem sido pesquisado infecção pelo Mycobaterium leprae *em tatus provenientes do Estado do Espírito Santo-Brasil. O teste rápido ML Flow, foi realizado em 37 tatus selvagens, tendo sido positivo em 11 (29,7%). O teste de ML Flow pode ser utilizado para identificar possíveis fontes de* Mycobaterium leprae *em tatus selvagens.*

Palavras-chaves: *Hanseníase. Teste ML Flow.* ***Reservatório. Tatus.***

Over the years, human beings have been considered to be the only reservoir of *Mycobacterium leprae*. However, leprosy in wild armadillos of the species *Dasypus novemcinctus* (nine-banded armadillo) was first reported in 1975[14], and natural transmission among armadillos in the southern parts of the United States has been described[13]. Some countries in Central and South America, such as Mexico, Argentina and Brazil, have reported leprosy occurring naturally in wild armadillos[1 6 9].

It seems that there is a need for increased knowledge on leprosy in wild armadillos, on order to establish whether or not armadillos are a source of *M. leprae* in Brazil, considering that there is some strong evidence supporting an association between human leprosy and contact with armadillos in some endemic areas, among patients in the United States[2 8], Mexico[11] and Brazil[5 10]. The origin, range and risks of armadillo leprosy remain unclear. Other than humans, armadillos are the only other natural leprosy hosts with high rates of the disease[13 14].

We have been investigating *Mycobacterium leprae* infection in armadillos since 1999. Our survey was conducted on armadillos captured from many rural parts of the State of Espírito Santo, Brazil. In this state, nine-banded armadillos are frequently slaughtered and eaten[4]. This region has high detection and prevalence rates for leprosy, and nine-banded armadillos are common and often come into contact with humans and other animals, direct and indirectly[5].

Several techniques are useful for diagnosing leprosy in armadillos. The technique with highest sensitivity is PCR, followed by serology with ELISA, necropsy and histopathology[7]. Positive serology was found in 16% of 565 armadillos from Louisiana (United States)[12] and positive PCR in 52.8%[7]. Truman *et al* showed a positive cross-reaction between the IgM antibodies of humans and armadillos, using ELISA, and detected the antigen PGL-I (phenolic glycolipid-I), which is specific for *M. leprae*[12]. Recently, a simple and rapid immunochromatographic flow test (the ML Flow test) became available for detecting PGL-I and IgM[3]. The sensitivity of

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the ML Flow test among multibacillary patients has been found to be 97.4% and the specificity to be 90.2%. It does not require any special equipment and it is suitable for use in the field, particularly in tropical countries[3]. We did not find any reports on the use of the ML Flow test on armadillos in any language.

The test was performed on 37 animals by the addition of 5µl of the whole blood or serum samples followed by the addition of 130µl of running buffer (phosphate-buffered saline containing 0.66mg of BSA and 3% Tween 20). The test result was read after 10 minutes, in accordance with the method of Bührer-Sékula *et al* [3]. The ML Flow test gave positive results in 11 of these nine-banded armadillos (29.7%): +2 in 4 animals, +1 in 7.

In human beings, the ML Flow test has been found to have a good correlation with ELISA (91%; κ=0.77)[3], and therefore it has been used to detect nine-banded armadillos as a possible source of *M. leprae*. The discovery of natural transmission among armadillos in the southern part of the United States suggested the possibility that nine-banded armadillos play a role in the transmission of human leprosy in the United States[14].

The ML Flow test seems to be suitable as a first test for investigating *M. leprae* in armadillos, and necropsy would only be done in positive cases. These preliminary results suggest that, in the State of Espírito Santo, nine-banded armadillos may be considered to be natural reservoir for *M. leprae*. The intention of the Leprosy Elimination Program is to detect all possible reservoirs of *M. leprae*, such as multibacillary patients and sources within the environment[12].

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